59. *The Configuration of Glucosamine (Chitosamine).*

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Chitosamine, the amino-hexose from chitin, has been alternatively described as glucosamine or mannosamine. Numerous attempts to solve this problem of configuration have failed because of the Walden inversion which occurs under certain conditions of deamination to the hexose. The investigation which follows has been designed to eliminate, by a synthetic procedure, one of the alternatives, mannose or glucose, as the parent sugar of chitosamine. The method has been to prepare a dimethyl 2 : 3-anhydromethylmannoside which has been shown to give rise, with alkali or sodium methoxide, to both a glucose derivative and an altrose derivative. The opening of the anhydro-ring in the dimethyl 2 : 3-anhydromethylmannoside was then carried out by the agency of ammonia, which gave rise to a derivative of 3-amino-altrose on the one hand and of 2-amino-glucose on the other. The latter was shown to be identical with chitosamine, which may now be considered configurationally to be glucosamine and therefore related to the parent sugar glucose.

The synthesis of glucosamine by Fischer and Leuchs (*Ber.*, 1903, **36**, 24) established its constitution as being that of a 2-amino-hexose but left open the question of the configuration at the second carbon atom. Many investigations were directed in the succeeding period towards a solution of this problem but a direct chemical approach has not hitherto been possible.

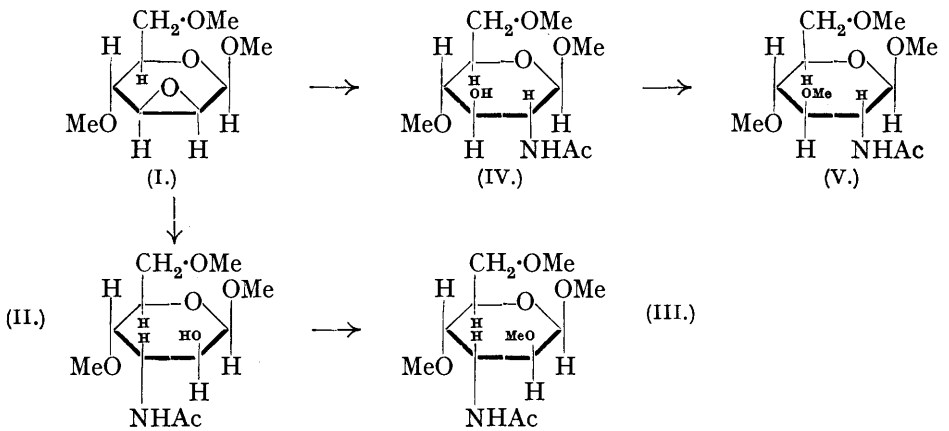
Consequent upon studies carried out in this laboratory on the properties of anhydro-sugars we have been able to devise a synthetic method which distinguishes between the alternative configurations (glucose or mannose) for glucosamine and which indeed proves that glucosamine belongs, in a configurational sense, to the glucose, and not to the mannose series. It has been shown that the fission by alkali (sodium methoxide or sodium hydroxide) of the ethylene oxide ring in anhydro-sugars leads to the formation of two isomeric sugars, consequent upon the independent rupture of the two bonds of the oxide oxygen atom. In each case the ring opening is accompanied by a Walden inversion on that carbon atom of the ethylene oxide ring at which the break occurs. Thus 3 : 4-anhydroallose gives a mixture of glucose and gulose (Peat and Wiggins, J., 1938, 1088); 2 : 3-anhydroallose, a mixture of glucose and altrose (*idem, ibid.*, p. 1810). A mixture of glucose and altrose is obtained also from 2 : 3-anhydromannose (Lake and Peat, J., 1938, 1417). Furthermore, when the alkaline agent is sodium methoxide, a methoxyl group becomes attached to the carbon atom at which Walden inversion occurs. Thus, to quote only one example of what seems to be a general rule, 2 : 6-dimethyl 3 : 4-anhydro-β-methylalloside, when boiled with sodium methoxide, gives a mixture of 2 : 3 : 6-trimethyl β-methylglucoside and 2 : 4 : 6-trimethyl β-methylguloside (J., 1938, 1088).

The fission of the anhydro-ring may also be effected by the action of ammonia, the same sequence of events being observed here as with sodium methoxide. Two amino-sugars are formed, the amino-group becoming attached in each case to that carbon atom at which Walden inversion occurs. For example, 2 : 3-anhydroallose gives 2-amino-altrose

and 3-amino-glucose. That the glucose derivative is correctly described as 3-amino-glucose and not as 2-amino-glucose follows from the fact that the same compound is produced by the action of ammonia on 3 : 4-anhydroallose (J., 1938, 1810). These considerations suggest a method for the synthesis of a derivative of 2-amino-glucose. Such a synthesis would, if successful, finally remove all dubiety concerning the configuration of naturally occurring glucosamine.

It was shown in a previous paper (Lake and Peat, *loc. cit.*) that 4 : 6-dimethyl 2 : 3-anhydro-β-methylmannoside behaves normally towards alkaline reagents and gives, when treated with sodium methoxide, a mixture of 2 : 4 : 6-trimethyl β-methylglucoside and 3 : 4 : 6-trimethyl β-methylaltroside. The constitution of each of the trimethyl methylhexosides was established by oxidative methods and no doubt exists that the methoxyl group has entered at position 3 in the altrose product and at position 2 in the glucose. If the behaviour of the anhydro-compound towards ammonia is also normal, then a mixture of derivatives of 2-amino-glucose and 3-amino-altrose is to be expected.

This expectation has been realised. A syrup having the composition of a dimethyl amino-methylhexoside was obtained in quantitative yield when 4 : 6-dimethyl 2 : 3-anhydro-β-methylmannoside (I) was heated under pressure with anhydrous methyl-alcoholic ammonia. The *N*-acetyl derivative of this product crystallised completely and by fractional crystallisation it was found possible to separate this derivative into two isomers (A) and (B). Fraction A (m. p. 150°) constituted 90% of the whole and fraction (B) (m. p.



187°) 10%. It was established, in the first place, that (A) was a derivative (II) of 3-amino-altrose. The free hydroxyl group of (II) was methylated by treatment of the substance with methyl iodide and silver oxide, and the product (III) shown to be identical with fully methylated *N*-acetyl *epi*-glucosamine. The latter substance was prepared from the methyl *epi*-glucosamine hydrochloride of Bodycote, Haworth, and Hirst (J., 1934, 151). These authors showed conclusively that the amino-group in *epi*-glucosamine is situated on C_3 and it follows that fraction (A) is also a derivative of a 3-amino-hexose. Moreover, since the amino-group has entered at C_2, it is clear, from the principles enunciated above, that the introduction of the amino-group will have been accompanied by a Walden inversion on C_3 and that (A) is correctly described as 4 : 6-*dimethyl* 3-*acetamido*-β-*methyl*-d-*altropyranoside* (II).

The recognition of fraction (A) as a derivative of 3-amino-altrose necessarily implies that the smaller fraction (B) is a derivative of 2-amino-glucose and is in fact 4 : 6-*dimethyl* 2-*acetamido*-β-*methyl*-d-*glucopyranoside* (IV). It is to be observed that there is a quantitative difference in the action of sodium methoxide and of ammonia on dimethyl 2 : 3-anhydro-methylmannoside. With the first reagent the altrose and glucose derivatives are formed in equal amounts, but with ammonia, the altrose member of the pair greatly preponderates. The methylation of (IV) gave 3 : 4 : 6-trimethyl 2-acetamido-β-methyl-*d*-glucopyranoside (V). This substance proved to be identical in properties (m. p. 195—

196°; $[\alpha]_D^{20°}$ − 30° in water and + 19° in chloroform) with the substance described by Cutler, Haworth, and Peat (J., 1937, 1979) as *N*-acetyl trimethyl β-methylglucosaminide (m. p. 195°; $[\alpha]_D^{16°}$ − 29° in water and $[\alpha]_D^{21°}$ + 19·6° in chloroform) and prepared by them from glucosamine by acetylation and methylation. The identity of the two products was confirmed by mixed m. p. determination, no depression being observed.

This result has two main consequences. It proves that the derivatives of glucosamine described by Cutler, Haworth, and Peat (*loc. cit.*) have the pyranose structure and it establishes, beyond reasonable doubt, that the biologically important hexosamine, glucosamine or chitosamine, is configurationally related to *d*-glucose and not to *d*-mannose.

A further point deserves attention. The description of fraction (B) (above) as a 2-amino-hexose does not depend entirely upon analogy. That the amino-group must be attached in position 2 follows from the fact that (V) is identical with the product from glucosamine and the latter is certainly a 2-amino-hexose. The entry of the amino-group in this position when the ethylene oxide ring is disrupted indicates that Walden inversion takes place on this carbon atom and the product (IV) has therefore the configuration of glucose.

Experimental.

The Action of Ammonia on 4 : 6-*Dimethyl* 2 : 3-*Anhydro*-β-*methylmannoside.*—The anhydro-compound (m. p. 69°; $[\alpha]_D^{19°}$ + 24° in water) (1·25 g.) was dissolved in dry methyl alcohol (50 c.c.) which had been saturated with ammonia at 0°, and the solution heated in a sealed tube at 130° for 30 hours. The product after removal of solvent was a viscous syrup (1·28 g.) having the composition of a dimethyl methylhexosaminide. After distillation at 125°/0·02 mm. it showed $n_D^{22°}$ 1·4780 and $[\alpha]_D^{21°}$ − 103° in methyl alcohol (*c*, 2·84) (Found: OMe, 41·8%; *M*, by van Slyke estimation of amino-group, 227. Calc. for $C_9H_{19}O_5N$: OMe, 42·1%; *M*, 221). A quantity of the syrupy amino-methylhexoside was accumulated by this method.

Crystalline derivatives were not formed when the dimethyl amino-methylhexoside (0·51 g.) was boiled with acetic anhydride and fused sodium acetate. The product (0·69 g.) was a very viscous syrup which distilled unchanged at 185°/0·004 mm. It had the composition of a *dimethyl amino-methylhexoside diacetate* (Found: OMe, 30·3. $C_{13}H_{23}O_7N$ requires OMe, 30·5%).

Preparation of Dimethyl Acetamido-methylhexoside.—To a cooled solution of the dimethyl amino-methylhexoside (0·83 g.) in dry methyl alcohol (15 c.c.) was added acetic anhydride (2 c.c.), and the mixture kept at room temperature for 12 hours. Thereafter the solution was diluted with water, neutralised with sodium bicarbonate, and evaporated to dryness at 45°. The solid residue was extracted with chloroform, the extract dried with anhydrous magnesium sulphate, and the solvent removed from it under diminished pressure. The product was a thick syrup which crystallised completely after distillation at 184° (bath temp.)/0·005 mm.

The same product was obtained when the syrupy diacetate (see above) was treated in methyl-alcoholic solution with a trace of sodium (this method removed only the *O*-acetyl). The dimethyl acetamido-methylhexoside so prepared is a mixture of 4 : 6-*dimethyl* 3-*acetamido*-β-*methyl*-d-*altropyranoside* (II) and 4 : 6-*dimethyl* 2-*acetamido*-β-*methyl*-d-*glucopyranoside* (IV). Fractional crystallisation was carried out in ethyl acetate and ethyl acetate–light petroleum. The most suitable solvent was later found to be chloroform–light petroleum. In this way 8·64 g. of the dimethyl acetamido-methylhexoside (from 7·56 g. of the dimethyl amino-methylhexoside; 96% yield) was separated into two fractions (A) and (B).

Fraction (A) (altrose derivative) formed prismatic crystals, m. p. 150°; $[\alpha]_D^{19°}$ − 108·0° in methyl alcohol (*c*, 3·2); yield, 7·7 g. (89% of total) (Found: C, 49·9; H, 8·0; N, 5·7; OMe, 35·2. $C_{11}H_{21}O_6N$ requires C, 50·2; H, 8·0; N, 5·3; OMe, 35·3%).

Fraction (B) (glucose derivative) crystallised in fine needles, m. p. 187°; $[\alpha]_D^{16°}$ − 21·5° in methyl alcohol (*c*, 2·48); yield, 0·8 g. (9·4% of total) (Found: C, 50·2; H, 8·1; N, 5·2; OMe, 35·1%).

2 : 4 : 6-*Trimethyl* 3-*acetamido*-β-*methylaltroside* (III) was obtained when fraction (A) (0·48 g.) was methylated by three treatments with Purdie's reagents. It distilled at 160° (bath temp.)/0·01 mm. as a viscid syrup which crystallised on trituration with ether. The crystals separated from ethyl acetate in large colourless prisms, m. p. 116°; $[\alpha]_D^{20°}$ − 97·7° in chloroform (*c*, 3·52); $[\alpha]_D^{20°}$ − 87·0° in water (*c*, 2·67); $[\alpha]_D^{21°}$ − 83·0° in methyl alcohol (*c*, 1·98). Yield, 0·3 g. (Found: C, 52·2; H, 8·5; N, 5·3; OMe, 44·4. $C_{12}H_{23}O_6N$ requires C, 52·0; H, 8·3; N, 5·1; OMe, 44·7%). This product was accompanied by a small amount of a mobile oil, b. p. 130° (bath temp.)/0·01 mm., the nature of which is being investigated.

Methylation of Fraction (*B*).—The methylation was accomplished by three treatments with methyl iodide and silver oxide, the presence of a litle acetone being advantageous. The product, 3 : 4 : 6-trimethyl 2-acetamido-β-methyl-*d*-glucopyranoside (V), crystallised from ethyl acetate in needle clusters and showed m. p. 195—196°; $[\alpha]_D^{20°}$ — 30° in water (*c*, 0·82, micro-tube) and $[\alpha]_D^{20°}$ — 19° in chloroform (*c*, 1·86, micro-tube) (Found : C, 52·0; H, 8·4; N, 5·3; OMe, 44·4%). The substance showed no depression of m. p. in admixture with the *N*-acetyl trimethyl β-methylglucosaminide prepared by Cutler, Haworth, and Peat (*loc. cit.*) from natural glucosamine. The m. p. of a mixture of (II) and (IV) was *ca.* 164° (depression) and of a mixture of (III) and the product from glucosamine was 108° (depression).

Acetylation and Methylation of Methyl epi-*Glucosamine Hydrochloride*.—3-*p*-Toluenesulphonyl triacetyl β-methylglucoside was converted by the method of Bodycote, Haworth, and Hirst (*loc. cit.*) into methyl *epi*-glucosamine hydrochloride. The latter substance (0·35 g.) was treated in methyl-alcoholic solution (8 c.c.) with acetic anhydride (1 c.c.) and silver acetate (0·1 g.). After being shaken at room temperature for 12 hours, the solution was filtered and evaporated to small bulk at 30°. Addition of ether threw down a gelatinous precipitate, which was washed with ether to remove acetic anhydride, dissolved in methyl alcohol, and reprecipitated by the addition of ether. Repetition of this process gave a pure product, 3-acetamido-β-methyl-altroside, which crystallised in needles from methyl alcohol–ether; m. p. 169° (softening at 166°); $[\alpha]_D^{20°}$ — 123° in methyl alcohol; yield, 0·27 g. This material (0·15 g.) was methylated by four treatments with methyl iodide and silver oxide (methyl alcohol was added as solvent in the first three methylations). The product separated from ethyl acetate–petrol in prisms, m. p. 116° and $[\alpha]_D^{21°}$ — 81° in methyl alcohol (micro-tube). In admixture with the trimethyl 3-acetamido-β-methylaltroside prepared as above described, it showed no depression of melting point.

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